Supplementary Information for

The males of the parasitoid wasp, *Nasonia vitripennis*, can identify which fly hosts contain females.

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Legend for Table S1

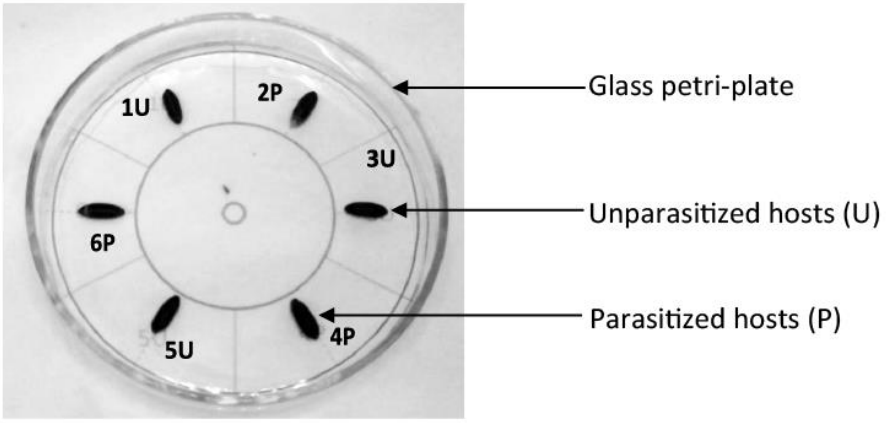

Figure S1. Cafeteria arena used for all the behavioural assays: The arena had two concentric circles (the outer 9 cm and the inner 5 cm diameter, respectively) divided into six equal chambers printed on a white sheet of paper over which a glass Petri plate (sterilized with ethanol, then with HPLC grade n-hexane and autoclaved) was placed. Autoclaved distilled water was added along the circumference to prevent males from escaping. This setup was placed on a wooden platform with a 5-watt LED lamp placed 30 cm above it. Each male was dropped at the centre of this arena, and the behaviour was recorded for 4 minutes by a video camera (Logitech C615 HD webcam) at 25°C ±1°C.

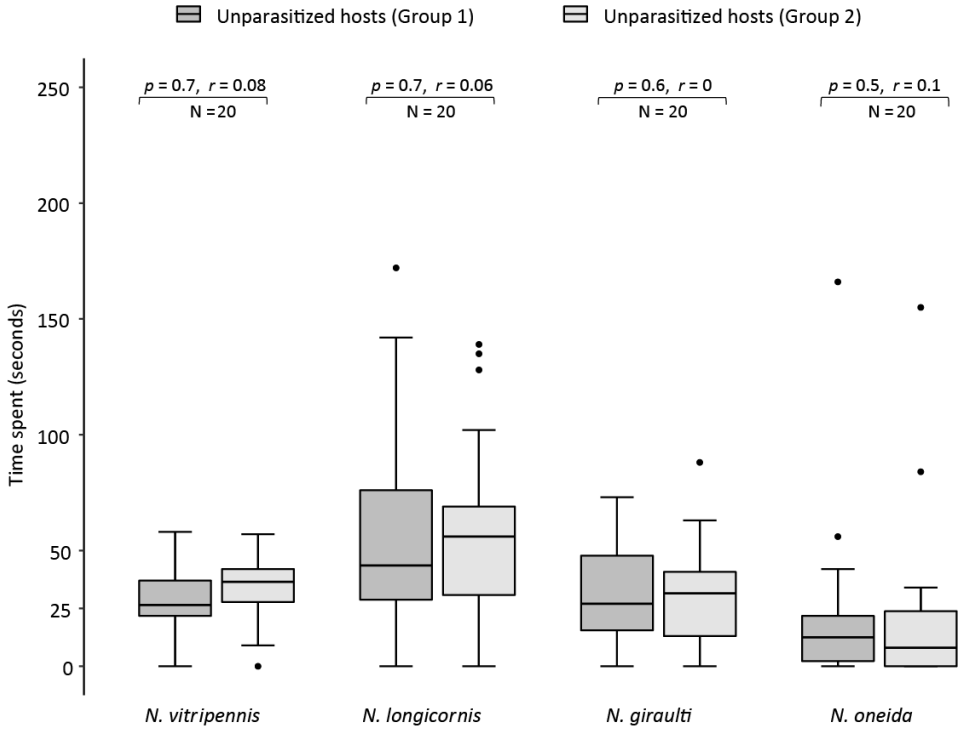

Figure S2. No inherent directional bias of male *Nasonia* in the cafeteria arena: To check whether *Nasonia* have an inherent bias towards any one direction within the arena, males were given a choice between six hosts (all unparasitized) and scored for time spent on each. These six unparasitized hosts were randomly divided into 2 groups of three each, to mimic the actual choice assay (as in figure S1), with the help of random numbers assigned to each (from 0 to 1). Random numbers with the lowest three values for each assay (N = 20) were assigned to "group 1" and the remaining to "group 2". Wilcoxon- signed-rank test was performed for assessing the difference in distribution of the two groups of unparasitized hosts. As the figure above indicates, no significant difference was found for the two groups across all the four species, indicating no evidence of a directional bias.

The numbers above the boxes represent the *p*-value and the sample size (N) for each species. In boxplots, the horizontal bold line within each box represents the median, boxes represent 25% and 75% quartiles, whiskers denote 1.5 interquartile ranges and black dots depict outliers. Statistical significance levels shown are according to Wilcoxon signed-rank test (statistically significant at $p < 0.05$) with ($*$) denoting a significant *p*-value. Wilcoxon effect size (*r*) values range from $r = 0.1$ - < 0.3 (small effect), $r = 0.3$ - < 0.5 (moderate effect) and $r >= 0.5$ (large effect). Species names are given at the bottom.

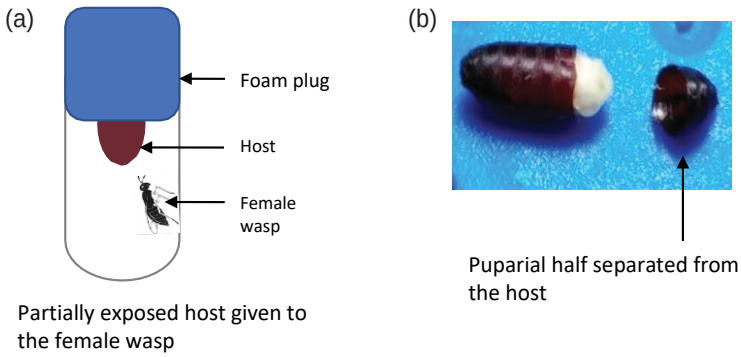

Figure S3. Use of puparial halves for the assays: A) Individual unparasitized host was half embedded in foam plugs put in vials so that only a part (anterior part) of the host was accessible to the female for parasitization. Half of such vials were exposed to individual mated female wasps while others remained female free, hence, unparasitized. B) After 48 hours, the hosts were taken out from plugs, carefully cracked open from the anterior part and the puparium separated from it. The hosts that were exposed to mated females were checked for the presence of eggs (now localized at the head of the fly pupa), and those found without eggs were discarded. The puparia of HwAMF and hosts containing adult fly (ten-day old) were collected after confirming the presence of adult wasps and adult fly, respectively.

Table - S1: List of all identified CHC compounds: There are 53 identified CHCs in HwAMF and HwAM and 47 in adult males and females (N = 4 for each sample). Each value represents mean relative abundance (± SEM or standard error of mean) expressed as a percentage. Retention times mentioned are of the samples HwAMF and HwAM (for chromatograms see figure 5).

S.No.	Retention Time	Linear Retention Index	Compound name	Mean relative abundance (%) ± SEM			
				HwAM	HwAMF	Adult male	Adult female
1	60.6	2500	C25	9.40 ± 0.58	14.88 ± 2.27	4.35 ± 0.35	12.72 ± 0.25
2	61.1	2534	MeC25 (9-; 11-; 13-)	0.73 ± 0.19	2.57 ± 0.15	-	-
3	61.6	3041	MeC25 (5-)	1.72 ± 0.30	3.04 ± 0.41	2.96 ± 0.26	8.08 ± 0.26
4	62	3141	MeC25 (3-)	1.29 ± 0.26	4.65 ± 0.29	1.70 ± 0.17	2.89 ± 0.09
5	62.5	2600	C26	0.87 ± 0.17	8.76 ± 2.07	0.91 ± 0.08	7.89 ± 0.12
6	62.66	3355	DiMeC26 (3,9-; 3,11-; 3,13; 3,15-)	0.87 ± 0.18	2.35 ± 0.05	-	-
7	63.15	2635	MeC26 (15-; 13-; 11-)	0.54 ± 0.09	1.70 ± 0.17	-	-
8	64.4	2700	C27	46.42 ± 0.51	43.62 ± 3.14	8.83 ± 0.59	27.30 ± 0.63
9	64.9	2735	MeC27 (15-;13-;11-;9-)	8.61 ± 0.73	14.05 ± 0.21	0.82 ± 0.06	1.69 ± 0.16
10	65.07	3149	MeC27 (7-)	1.36 ± 0.11	2.32 ± 0.10	0.67 ± 0.08	1.74 ± 0.05
11	65.25	3173	MeC27 (5-)	2.82 ± 0.29	4.48 ± 0.33	2.59 ± 0.16	5.04 ± 0.06
12	65.66	3244	MeC27 (3-)	13.15 ± 0.70	17.55 ± 0.94	4.84 ± 0.37	8.75 ± 0.20
13	65.8	2785	DiMeC27 (5, x-)	1.28 ± 0.06	1.21 ± 0.11	0.54 ± 0.10	0.93 ± 0.09
14	66.1	2800	C28	2.76 ± 0.35	9.74 ± 1.51	1.50 ± 0.07	7.97 ± 0.30
15	66.25	3365	DiMeC27 (3,9-;3,11-;3,13-; 3,15-)	5.47 ± 0.50	7.92 ± 0.26	1.52 ± 0.12	3.38 ± 0.27
16	66.67	2832	MeC28 (9-; 11-; 12-; 13-; 14-; 15)	3.68 ± 0.46	3.88 ± 0.39	-	-
17	67.1	3340	MeC28 (4-)	0.99 ± 0.16	2.08 ± 0.15	-	-
18	67.85	2900	C29	100.00 ± 0	88.01 ± 1.11	53.79 ± 1.71	63.47 ± 1.26
19	68.35	2932	MeC29 (15-;13-;11-)	27.88 ± 1.21	27.28 ± 0.49	5.31 ± 0.21	7.06 ± 0.33
20	68.5	3349	MeC29 (7-)	10.62 ± 0.69	10.94 ± 0.23	12.81 ± 0.64	29.72 ± 0.03
21	68.65	3373	MeC29 (5-)	10.56 ± 0.62	13.74 ± 0.29	10.64 ± 2.47	17.32 ± 0.36
22	69.05	3537	MeC29 (3-)	43.45 ± 0.98	40.60 ± 0.52	8.75 ± 0.38	13.12 ± 0.42
23	69.17	2987	DiMeC29 (5,x-)	3.44 ± 0.08	2.09 ± 0.12	1.48 ± 0.12	5.53 ± 0.20
24	69.45	3000	C30	6.56 ± 0.52	13.06 ± 0.72	5.00 ± 0.16	6.44 ± 0.52
25	69.58	3395	DiMeC29 (3,11-; 3,13-; 3,15-)	10.26 ± 0.30	5.74 ± 0.31	1.24 ± 0.03	1.98 ± 0.20
26	70	3560	MeC30 (7-; 8-)	6.96 ± 0.53	4.97 ± 0.26	1.73 ± 0.19	2.88 ± 0.59

S.No.	Retention Time	Linear Retention Index	Compound name	Mean relative abundance (%) ± SEM			
				HwAM	HwAMF	Adult male	Adult female
27	70.77	3453	C31-ene	4.19 ± 0.45	3.49 ± 0.23	1.43 ± 0.20	41.43 ± 2.54
28	71.15	3100	C31	98.98 ± 0.46	100.00 ± 0	100.00 ± 0	81.25 ± 1.11
29	71.65	3131	MeC31 (15-;13;11-)	26.90 ± 0.99	33.24 ± 0.54	39.54 ± 0.75	26.82 ± 1.15
30	71.85	2551	MeC31 (7-)	26.70 ± 0.89	25.37 ± 0.83	55.15 ± 1.02	100.00 ± 0
31	72	2573	MeC31 (5-)	11.13 ± 0.64	13.86 ± 0.38	23.09 ± 0.84	35.04 ± 0.46
32	72.3	3171	DiMeC31 (7,11-)	3.31 ± 0.43	2.60 ± 0.33	3.14 ± 0.13	7.66 ± 0.40
33	72.45	2740	MeC31 (3-)	14.60 ± 0.51	26.37 ± 1.28	17.61 ± 0.39	20.71 ± 1.36
34	72.57	3178	DiMeC31 (7,x-)	3.07 ± 0.21	2.84 ± 0.16	4.83 ± 0.39	18.54 ± 1.04
35	72.9	3200	C32	3.67 ± 0.30	7.44 ± 0.41	3.58 ± 0.13	4.92 ± 0.45
36	73.2	2750	MeC32 (6-)	2.62 ± 0.26	4.16 ± 0.29	4.20 ± 0.12	6.45 ± 0.17
37	74.1	3074	TetraMeC31 (3,7,11,15-)	1.28 ± 0.05	4.20 ± 0.26	3.71 ± 0.67	3.28 ± 0.72
38	75.05	3300	C33	29.64 ± 0.41	27.96 ± 1.58	2.60 ± 0.29	6.49 ± 0.09
39	75.7	3335	MeC33 (15-;13-;11-)	34.69 ± 0.55	42.78 ± 2.13	47.27 ± 3.08	31.86 ± 3.03
40	75.95	2775	MeC33 (7-)	4.38 ± 0.12	4.20 ± 0.21	7.50 ± 0.66	10.23 ± 0.15
41	76.22	2858	MeC33 (5-)	5.30 ± 0.22	7.78 ± 0.93	6.98 ± 0.35	5.47 ± 0.43
42	76.54	2810	DiMeC33 (11,15-;11,21)	14.23 ± 0.52	16.59 ± 0.56	25.45 ± 2.51	10.33 ± 1.55
43	76.7	3012	DiMeC33 (7,19-; 7,23-)	5.26 ± 0.18	5.71 ± 0.40	11.09 ±0.31	11.13 ±0.99
44	76.85	2939	MeC33 (3-)	22.41 ± 0.83	27.03 ± 1.13	10.05 ± 1.22	21.64 ± 1.12
45	77.6	2610	DiMeC33 (3,17-; 3,15-)	5.79 ± 0.14	11.30 ±1.40	10.50 ± 1.18	11.92 ±1.38
46	79.25	3260	TetraMeC33 (3,7,11,15-)	6.57 ± 0.19	12.06 ± 1.26	9.48 ± 1.48	6.74 ± 0.91
47	80.4	3500	C35	3.38 ± 0.13	2.68 ± 0.34	-	-
48	81.3	3524	MeC35 (17-)	18.59 ± 0.80	22.02 ± 2.42	14.03 ± 2.16	12.06 ± 1.82
49	82.2	3532	MeC35 (15-;13-;11-)	26.47 ± 1.73	40.08 ± 4.83	31.74 ± 4.33	12.63 ± 2.08
50	82.7	2950	MeC35 (7-)	5.50 ± 0.99	6.22 ± 0.42	9.41 ± 1.15	15.80 ± 1.93
51	83.05	2971	MeC35 (5-; 3-)	16.31 ± 1.72	21.11 ±	2.46	10.57 ± 1.89
52	89.3	3730	MeC37 (17-)	1.96 ± 0.05	3.01 ± 0.51	2.55 ± 0.45	2.85 ± 0.41
53	90.5	3722	MeC37 (15-; 13-)	8.65 ± 0.48	16.34 ± 3.04	7.88 ± 0.70	3.82 ± 0.48

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